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Kay Bulen (Name) Kay Bulen (Signature) June 22, 2001 (Date)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Mark L. Gonzalgo et al.
Parent Serial No.:	09/094,207
Parent Filed:	09 June 1998
For:	CANCER DIAGNOSTIC METHOD BASED UPON DNA METHYLATION DIFFERENCES
Parent Examiner:	C. Myers
Parent Art Unit:	1655
Docket No.:	47675-21
Date:	22 June 2001

Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

PRELIMINARY AMENDMENT

Madam:

This is a Preliminary Amendment to accompany a Continuation Patent Application, under 37 C.F.R. § 1.53(b), for USSN 09/094,207 filed 09 June 1998. Kindly amend the above-identified continuation patent application as follows:

IN THE SPECIFICATION:

Pursuant to 37 C.F.R. § 121(b), please substitute the following paragraph for the corresponding paragraph on page 1, lines 4-6. Appendix A (attached hereto) contains a "marked up" version of this paragraph, showing applicants' present amendments to the originally-submitted text.

"Clean paragraph":

This patent application is a continuation of United States Patent Application 09/094,207 filed 09 June 1998, which claims priority from United States Provisional Patent Application 60/049,231 filed 09 June 1997.

IN THE CLAIMS:

Please cancel original claims 1-10, and, pursuant to 37 C.F.R. § 121(c), submit therefore new claims 11-22 as follows:

We claim:

11. (New) A method for determining DNA methylation status at a cytosine residue of a CpG sequence, comprising the steps of:

(a) obtaining genomic DNA from a DNA sample to be assayed;

(b) reacting the genomic DNA with sodium bisulfite to convert unmethylated cytosine residues to uracil residues while leaving any 5-methylcytosine residues unchanged to create an exposed bisulfite-converted DNA sample having binding sites for primers specific for the bisulfite-converted DNA sample;

(c) performing a PCR amplification procedure using top strand or bottom strand specific primers;

(d) isolating the PCR amplification products;

(e) performing a primer extension reaction using a Ms-SNuPE primer, labeled dNTPs and *Taq* polymerase, wherein the Ms-SNuPE primer comprises from about a 15 mer to about a 22 mer length primer sequence that is complementary to the bisulfite-converted DNA sample and terminates immediately 5' of the cytosine residue of the CpG sequence to be assayed; and

(f) determining the methylation status at the cytosine residue of the CpG sequence by measuring the incorporation of the labeled dNTPs.

12. (New) The method of claim 11 wherein the dNTPs are labeled with a label selected from the group consisting of radiolabels, fluorescent labels, phosphorescent labels, enzymic labels, mass labels detectable in a mass spectrometer, and combinations thereof.

13. (New) The method of claim 11 wherein the labeled dNTP for top strand analysis is labeled dCTP or labeled TTP.

14. (New) The method of claim 11 wherein the labeled dNTP for bottom strand analysis is labeled dATP or labeled dGTP.

15. (New) The method of claim 11 wherein the isolation step of the PCR products uses an electrophoresis technique.

16. (New) The method of claim 15 wherein the electrophoresis technique uses an agarose gel.

17. (New) The method of claim 11 wherein the Ms-SNuPE primer sequence comprises a sequence of at least fifteen but no more than twenty five nucleotides of a sequence selected from the group consisting of GaL1 [SEQ ID NO:1], GaL2 [SEQ ID NO:2], GaL4 [SEQ ID NO:3], HuN1 [SEQ ID NO:4], HuN2 [SEQ ID NO:5], HuN3 [SEQ ID NO:6], HuN4 [SEQ ID NO:7],

HuN5 [SEQ ID NO:8], HuN6 [SEQ ID NO:9], CaS1 [SEQ ID NO:10], CaS2 [SEQ ID NO:11], CaS4 [SEQ ID NO:12], and the bisulfite-converted equivalents thereof.

18. (New) A Ms-SNuPE primer that terminates immediately 5' upstream of a cytosine residue in a CpG sequence of a CpG island that is frequently hypermethylated in promoter regions of somatic genes in malignant tissue, wherein said Ms-SNuPE primer comprises an oligonucleotide consisting of at least 15 contiguous nucleotides of a gene sequence located immediately 5' upstream from the CpG sequence.

19. (New) The Ms-SNuPE sequence of claim 18 wherein the primer sequence is from about 15 to about 25 nucleotides in length and selected from the group consisting of GaL1 [SEQ ID NO:1], GaL2 [SEQ ID NO:2], GaL4 [SEQ ID NO:3], HuN1 [SEQ ID NO:4], HuN2 [SEQ ID NO:5], HuN3 [SEQ ID NO:6], HuN4 [SEQ ID NO:7], HuN5 [SEQ ID NO:8], HuN6 [SEQ ID NO:9], CaS1 [SEQ ID NO:10], CaS2 [SEQ ID NO:11], CaS4 [SEQ ID NO:12], and the bisulfite-converted equivalents thereof.

20. (New) A method for obtaining a Ms-SNuPE primer sequence that terminates immediately 5' of a cytosine residue in a CpG sequence of a CpG island, comprising finding a hypermethylated CpG sequence in a CpG island in a somatic gene from a malignant tissue or cell culture, determining the sequence located immediately 5' upstream from the hypermethylated CpG sequence, and synthesizing, based at least in part on using the sequence located immediately 5' upstream from the hypermethylated CpG sequence as a template, a Ms-SNuPE primer comprising a 15 to 25 nucleotide sequence immediately 5' upstream from the hypermethylated CpG sequence.

21. (New) A Ms-SNuPE primer comprising a 15 to 25 mer oligonucleotide sequence obtained by the process comprising finding a hypermethylated CpG sequence in a CpG island in a somatic gene from a malignant tissue or cell culture, determining the sequence located immediately 5' upstream from the hypermethylated CpG sequence, and synthesizing, based at least in part on using the sequence located immediately 5' upstream from the hypermethylated CpG sequence as a template, a Ms-SNuPE primer comprising a 15 to 25 nucleotide sequence immediately 5' upstream from the hypermethylated CpG sequence, wherein said primer terminates immediately 5' upstream from the hypermethylated CpG sequence.

22. (New) The method of claim 11, wherein performing a primer extension reaction comprises simultaneous use of a plurality of unique MS-SNuPE primers, and wherein each primer comprises from about a 15 mer to about a 22 mer length primer sequence that is complementary to the bisulfite-converted DNA sample and terminates immediately 5' of one of a plurality of unique CpG sequences, whereby the relative methylation status of the plurality of unique CpG sequences can be simultaneously determined.

REMARKS

Applicants respectfully request consideration of new claims 11-22 of the above-identified patent application to provide for completion of claims supported by the original specification filed on 09 June 1997. The present claims of this continuation patent application provide for subject matter supported in the specification and providing the full scope of the invention as conceived by the inventors, and in view of the knowledge and ordinary skill in the art at the time of filing.

No new matter has been added.

Entry of the foregoing amendment is respectfully requested. Claims 11-22 are pending.

Respectfully submitted,

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APPENDIX A

("marked up" paragraph, showing applicants' present amendments to originally-submitted text)

This patent application is a continuation of United States Patent Application 09/094,207 filed 09 June 1998, which claims priority from United States Provisional Patent Application 60/049,231 filed 09 June 1997.